

Characterization of Cases of *Clostridium difficile* Infection (CDI) Presenting at an Emergency Room: Molecular and Clinical Features Differentiate Community-Onset Hospital-Associated and Community-Associated CDI in a Tertiary Care Hospital[▽]

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Definition of community-onset, hospital-acquired *Clostridium difficile* infection (CO-HA-CDI) is difficult in patients presenting with diarrhea at hospitals or outpatient clinics, especially 4 to 12 weeks after the last discharge. We performed *C. difficile* stool culture for 272 diarrheic patients visiting the emergency room (ER) between January 2006 and June 2010. *C. difficile* was isolated from 36 cases (13.2%), and isolation rates increased year by year, from 10.1% in 2008 to 12.4% in 2009 and 16.7% in 2010. Among 32 toxin-positive isolates, 13 (40.6%) and 19 (59.4%) were associated with CO-HA-CDI and community-acquired CDI (CA-CDI), respectively, if cases with CDI diagnosed within 12 weeks after discharge were considered hospital associated. The majority (70%) of CO-HA-CDI cases occurred within 2 weeks after hospital discharge, although the interval from discharge to onset of symptoms was as long as 10 weeks. We found via *tcdA* and *tcdB* and repetitive sequence PCR analysis, that toxin A-positive/toxin B-positive isolates were the most prevalent in both CO-HA-CDI (53.8%) and CA-CDI (94.7%) cases. Toxin A-negative/toxin B-positive isolates were also still highly associated with HA-CDI cases but were also observed in CA-CDI cases. Younger age, fewer underlying diseases, lack of prior antibiotic use, and genetic diversity of isolates in repetitive sequence PCR were the main characteristics in CA-CDI cases visiting the ER.

Clostridium difficile is the most important hospital-acquired pathogen responsible for antibiotic-associated diarrhea. Community-acquired *C. difficile* infection (CA-CDI) has been reported worldwide (1, 7, 9, 11, 24). However, it is not easy to differentiate CA-CDI from hospital-acquired CDI (HA-CDI), although recommendations have been published (10). According to CDI surveillance recommendations, CA-CDI is defined by symptom onset ≥ 12 weeks after the last discharge from a health care facility, with patients classified as having indeterminate disease if they were discharged during the previous 4 to 12 weeks (10). Cases are classified as community-onset, hospital-associated CDI (CO-HA-CDI) if they were discharged from a health care facility within the previous 4 weeks. However, other authors variously classify cases as CA-CDI if symptom onset occurs without hospitalization during the past 6 months, 100 days, 3 months, or 8 weeks (3, 9, 24). Therefore, it is still controversial to define CO-HA-CDI when patients with diarrhea visit a hospital or an outpatient clinic after hospital discharge, especially 4 to 12 weeks after discharge (3, 4). At that time, pathogens other than *C. difficile* may also be the etiologic agents of diarrhea (1, 15).

For several years, we have observed diarrhea and abdominal pain to be the chief complaints among patients arriving at the

emergency room (ER) at a tertiary care hospital. *C. difficile* culture has frequently been ordered at Sanggye Paik Hospital ER because we have had a high prevalence of CDI since 2004 and *C. difficile* culture was encouraged (17, 18). Since 2006, *C. difficile* culture-positive cases have been found in the ER, and the proportion of *C. difficile* cases among all diarrhea cases seemed to be increasing. Therefore, we retrospectively investigated *C. difficile*-positive cases presenting at the ER to establish guidelines by which to differentiate CA-CDI from CO-HA-CDI through molecular (PCR assay for toxin gene and genotyping) characterization of *C. difficile* isolates and from the clinical features of *C. difficile* culture-positive cases.

MATERIALS AND METHODS

Specimens. We cultured stool samples from 272 patients visiting the ER between January 2006 and June 2010 (2 in 2006, 12 in 2007, 69 in 2008, 153 in 2009, 36 from January to June 2010). We also cultured for *Salmonella* and *Shigella* spp. The Institutional Review Board of Sanggye Paik Hospital approved the study protocol.

***C. difficile* culture.** *C. difficile* culture was done as previously described (18). A fecal specimen (1.0 ml) was mixed with an equal volume of 70% isopropanol and incubated at room temperature for 30 min. One drop (~100 μ l) was then inoculated onto prereduced *Clostridium difficile* selective agar (CDSA; Becton Dickinson, Sparks, MD) and incubated at 37°C under anaerobic conditions (Anaerobic pouch; Becton Dickinson, Sparks, MD) for 48 to 72 h. Suspected *C. difficile* colonies were identified by spore staining and use of an ANA identification test kit (bioMérieux SA, Marcy l'Etoile, France).

Enzyme immunoassays (EIAs) for toxin A and/or toxin B. Stool specimens were examined for toxin A (2006 to 2007) and toxin A/B (2008 to 2010) via an enzyme-linked fluorescent immunoassay (Vidas; bioMérieux SA, Marcy l'Etoile, France), as described previously (19). Assay results were positive, negative, or

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TABLE 1. Demographic characteristics of *C. difficile* infection cases categorized by hospitalization history and time interval from hospital discharge to visiting the emergency room (symptom onset)

Characteristics ^a	<i>C. difficile</i> -positive cases	Hospitalized cases		Nonhospitalized cases or cases hospitalized for >12 wk	<i>P</i> value
		<4 wk	4–12 wk		
Total no. (%) of cases	32	9 (28.1)	4 (12.5)	19 (59.4)	
No. of M:no. of F	17:15	7:2	3:1	7:12	0.036
Mean (range) age (yr)	55 (19–92)	62 (30–78)	71 (47–92)	49 (18–81)	0.032
No. (%) of cases					
Antibiotic use	14 (43.8)	7 (77.8)	2 (50.0)	5 (26.3)	0.029
Underlying disease	24 (75.0)	9 (100)	4 (100)	11 (57.9)	0.010
EIA positive	17 (53.1)	5 (55.6)	3 (75.0)	9 (47.4)	0.524
Toxin A/B gene status					
<i>tcdA</i> ⁺ <i>tcdB</i> ⁺	25 (78.1)	4 (44.4)	3 (75.0)	18 (94.7)	
<i>tcdA</i> [−] <i>tcdB</i> ⁺	7 (21.9)	5 (55.6)	1 (25.0)	1 (5.3)	

^a M, male; F, female; *tcdA*⁺, *tcdA* positive; *tcdB*⁺, *tcdB* positive; *tcdA*[−], *tcdA* negative.

equivocal according to the fluorescence intensity, as described in the relevant package insert, for each assay.

PCR assay for *tcdA* and *tcdB* and binary toxin gene (*cdtA* and *cdtB*). PCRs for *tcdA* and *tcdB* were done on 39 *C. difficile* isolates (36 from ER patients and 3 stored *C. difficile* isolates from ER patients who had earlier episodes of CDI while hospitalized) as previously described (6, 18). The PCR product for *tcdA* was 1,200 bp if the gene was intact and 700 bp if it was the variant gene (6). PCR assays for binary toxin genes (*cdtA* and *cdtB*) were performed as previously described (22).

rep-PCR. For genotyping, *C. difficile* isolates (the same as those in the PCRs for *tcdA* and *tcdB*) were examined by repetitive sequence-based PCR (rep-PCR; DiversiLab, bioMérieux SA, Marcy l'Etoile, France), with minor modifications (14).

DNA was extracted from colonies using an UltraClean microbial DNA isolation kit (Mo Bio Laboratories), following the manufacturer's instructions, and diluted to 35 to 50 ng/μl. DNA was amplified using a DiversiLab Clostridium DNA fingerprinting kit, according to the manufacturer's instructions: 2 μl of genomic DNA, 18 μl of the rep-PCR master mix, 2 μl of primer mix, 0.5 μl of AmpliTaq polymerase, and 2.5 μl of 10× PCR buffer were added for a total of 25 μl per reaction mixture. Thermal cycling parameters for PCR included initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 70°C for 1.5 min and a final extension at 70°C for 3 min. Kit-supplied positive and negative controls were run with each reaction set. Rep-PCR products were detected using a DiversiLab system in which the DNA amplicons are separated using microfluidic lab-on-a-chip technology (LabChip device; Caliper Technologies) and a model 2100 bioanalyzer (Agilent Technologies). Analysis was performed with the web-based DiversiLab software (version 3.4.40).

PCR ribotyping. PCR ribotyping was done as previously described (13, 16). Amplifications were carried out in a thermal cycler (iCycler; Bio-Rad) for 1 cycle of 15 min at 95°C for initial heat activation, followed by 35 cycles (1 min at 94°C, 1 min at 55°C, 2 min at 72°C) and 1 cycle of 60 min at 75°C for concentration to a final volume of 25 μl. PCR products were detected by electrophoresis in 3% (wt/vol) pulsed-field-certified agarose (Bio-Rad) for 2 h 30 min at 9 V/cm in 0.5× TBE (Tris-borate-EDTA) buffer chilled at 4°C. The DNA banding pattern was visualized under UV light after the gel was stained for 30 min in ethidium bromide (0.5 μg/ml).

Classification of case. Chart review was conducted for culture-positive patients. The patients were divided into 3 groups, according to the hospitalization history and the time interval from hospital discharge. Patients who had been hospitalized within the 4 weeks prior to the onset of CDI symptoms were placed in group A (CO-HA-CDI). Patients with a history of hospitalization in the 4 to 12 weeks prior to symptom onset constituted group B (presumed CO-HA-CDI). Those who had symptom onset more than 12 weeks after the last discharge or no history of hospitalization made up group C (CA-CDI).

RESULTS

C. difficile was isolated from 36 of 272 diarrheic patients (13.2%) visiting the ER. The isolation rate increased year by

year from 2008 (10.1%) to 2009 (12.4%) to 2010 (16.7%). In 2006 and 2007, the numbers of cases were relatively fewer (2 and 12, respectively). No *Salmonella* or *Shigella* spp. were isolated.

PCR for *tcdA* and *tcdB* revealed that among 36 *C. difficile* isolates, the numbers of toxin A-positive/toxin B-positive (A⁺B⁺), toxin A-negative/toxin B-positive (A[−]B⁺), and toxin A-negative/toxin B-negative (A[−]B[−]) isolates were 25 (69.4%), 7 (19.4%), and 4 (11.2%), respectively. Among them, 2 (5.6%) A⁺B⁺ isolates yielded *cdtA* and *cdtB* genes (cases 19 and 29). In 32 toxin-positive CDI cases, 9 (28.1%), 4 (12.5%), and 19 (59.4%) were categorized in groups A, B, and C, respectively (Table 1). The median age of the patients in group C (49 years; range, 18 to 81 years) was lower than the medians in groups A (62 years; range, 30 to 78 years) and B (71 years; range, 47 to 92 years) (*P* = 0.032). Only 17 out of 32 CDI cases (53.1%) were toxin A and/or toxin B positive (15 cases) or equivocal (2 cases) via EIA. All members of group A visited the ER within 2 weeks after discharge and yielded toxigenic isolates (4 A⁺B⁺ and 5 A[−]B⁺). Binary toxin-producing A⁺B⁺ isolates belonged to group A. In group B, 3 cases yielded A⁺B⁺ isolates and an A[−]B⁺ isolate was recovered from the 1 other case. In group C, 14 cases had no hospitalization history. The median time from discharge to symptom onset was 39 months (range, 8 months to 6 years) in the other 5 cases with hospitalization histories. The majority (94.7%) of them had A⁺B⁺ isolates, although one had an A[−]B⁺ isolate. Therefore, among 7 A[−]B⁺ isolates, 6 (85.7%) were from patients in groups A and B (HA-CDI cases) and 1 was from a patient in group C (without a hospitalization history).

Histories of antibiotic use were observed in 77.8%, 50.0%, and 26.3% of patients in groups A, B, and C, respectively (*P* 0.029). Cephalosporins were the most frequently prescribed (80%). Underlying diseases were observed in 100% of group A and group B patients but in only 57.9% of those in group C. However, the rate was only 46.7% in group C patients if 5 cases with hospitalization histories were excluded. Malignancy, diabetes mellitus (DM), and hypertension were the most prevalent underlying diseases.

Rep-PCR analysis yielded 28 different profile groups (Fig. 1). One major cluster (5 isolates) and 4 minor clusters (2 isolates

Rep-PCR	No.	Year	Toxin	Ribotype	Hospitalization history (weeks after discharge)
	1	2010,03	A+B+	AB1	+ (260,7)
	2	2010,01	A-B-	ab1	+ (7,6)
	3	2009,11	A+B+	AB2	-
	4	2008,07	A+B+	AB2	+ (8,6)
	5	2010,04	A+B+	AB2	-
	6	2009,06	A+B+	AB3	+ (104,3)
	7	2007,12	A-B-	ab2	-
	8	2009,12	A+B+	AB4	+ (34,3)
	9	2009,09	A+B+	AB4	-
	10	2008,02	A-B+	aB	+ (0,9)
	10 -1*	2008,01	A-B+	aB	-
	11	2009,04	A+B+	AB5	-
	12	2009,08	A+B+	AB5	-
	13	2007,10	A+B+	AB5	+ (0,4)
	14	2006,02	A+B+	AB5	-
	15	2009,10	A-B-	ab1	+ (109,1)
	16	2009,07	A+B+	AB6	-
	17	2007,10	A+B+	AB7	-
	18	2009,08	A+B+	AB7	+ (6,3)
	19	2009,02	A+B+	AB8	+ (1,1)
	19 -1*	2009,01	A+B+	AB8	-
	20	2008,05	A-B+	aB	-
	21	2010,05	A-B+	aB	+ (1,7)
	22	2008,11	A+B+	AB7	-
	23	2009,12	A-B-	ab3	+ (10,0)
	24	2009,09	A+B+	AB9	-
	25	2008,03	A-B+	aB	+ (0,9)
	ATCC43598		A-B+		
	26	2009,01	A+B+	AB10	+ (1,6)
	27	2008,09	A+B+	AB10	+ (0,6)
	28	2010,03	A-B+	aB	+ (4,3)
	29	2009,03	A+B+	AB11	+ (139,0)
	ATCC43596		A+B+		
	30	2009,03	A-B+	aB	+ (1,6)
	31	2006,11	A-B+	aB	+ (2,0)
	32	2009,07	A+B+	AB12	-
	33	2009,02	A+B+	AB12	-
	34	2009,04	A+B+	AB12	+ (312,0)
	35	2009,03	A+B+	AB12	+ (10,0)
	35 -1*	2008,12	A+B+	AB12	-
	36	2008,03	A+B+	AB12	-

FIG. 1. Repetitive sequence-based PCR results for the 39 *C. difficile* isolates, including 3 isolates from the earlier hospitalization. A total of 28 different profile groups were found, and 1 major cluster (cases 32, 33, 34, 35, and 36) and 4 minor clusters (cases 3 and 4, 13 and 14, 26 and 27, and 30 and 31) were detected. Among A⁺B⁺ isolates ($n = 25$), 17 patterns were observed, and no identical patterns were seen in A⁻B⁻ isolates ($n = 4$). Among the 7 A⁻B⁺ isolates (cases 10, 20, 21, 25, 28, 30, and 31), only 2 isolates (cases 30 and 31) showed identical patterns. Isolates from three cases of recurrent CDI (cases 10, 19, and 35) showed rep-PCR patterns identical to those from the earlier episodes (*). PCR ribotyping revealed that 12, 1, and 3 patterns in A⁺B⁺, A⁻B⁺, and A⁻B⁻ isolates, respectively.

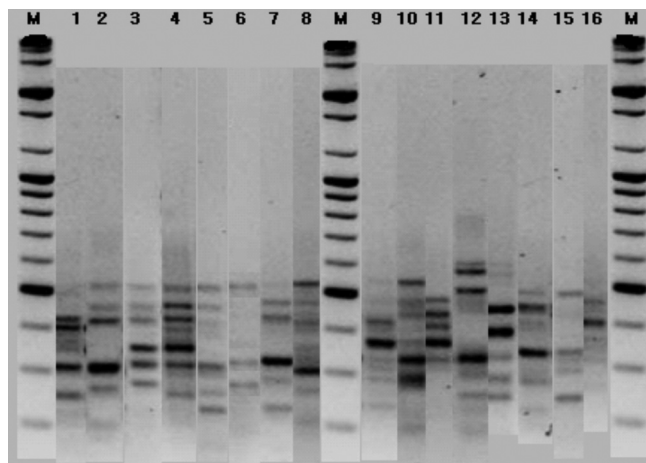


FIG. 2. PCR-ribotyping fingerprints of *C. difficile* isolates belonging to PCR ribotypes AB1 to AB12 (lane 1 to lane 12, respectively), aB (lane 13), and ab1 to ab3 (lane 14 to lane 16, respectively). Lanes M, 100-bp ladder.

in each cluster; 3 A^+B^+ clusters, 1 A^-B^+ cluster) were detected. In one major cluster, 3 patients had no hospitalization history and the other 2 patients had been hospitalized, but the time interval between them was 6 years. No associated factors, including address, ward of admission, and date of hospitalization, were observed among each of the major and minor clusters. Six distinct patterns were observed among 7 A^-B^+ isolates, and only 2 isolates showed identical patterns. No identical patterns were seen among A^-B^- isolates. PCR ribotyping revealed 12, 1, and 3 patterns among A^+B^+ , A^-B^+ , and A^-B^- isolates, respectively (Fig. 2). All of the A^-B^+ isolates had the same banding pattern as ATCC 43598 (PCR ribotype 017) by PCR-ribotyping. Some of the A^+B^+ isolates had identical banding patterns by PCR-ribotyping, which matched the rep-PCR patterns (cases 3 and 4, 13 and 14, 26 and 27, and 32, 33, 34, 35, and 36 in Fig. 1).

We found 3 cases of CDI (cases 10, 19, and 35 in Fig. 1) in group A ($n = 2$) and group B ($n = 1$) who had had earlier episodes of CDI while they were hospitalized. The intervals between the earlier episodes and the outpatient diagnosis were 21 days in case 10, 15 days in case 19, and 88 days in case 35. Identical rep-PCR patterns were found among isolates from the same patients.

DISCUSSION

We present new data about *C. difficile* in patients visiting the ER for gastroenteritis or colitis. One reason is that *C. difficile* culture is not routine for a patient with diarrhea and other gastroenteritis symptoms visiting the ER or a general practitioner. The other reason is that such cases are usually regarded as food poisoning or gastroenteritis caused by other endemic pathogens (such as *Shigella*, *Salmonella*, enterohemorrhagic *Escherichia coli*, and *Campylobacter* spp.).

CA-CDI is emerging in healthy young persons in the community (5, 12). CA-CDI frequently occurs without exposure to antibiotics and comprises about 20 to 22% of CDI cases (7, 12). It has also been reported that 4 to 20% of nursing home

residents and 3% of the healthy population are asymptomatic carriers of *C. difficile* (12, 20). The asymptomatic carriers could spread *C. difficile* in the community and in hospitals. Therefore, it is important to investigate the characteristics and patterns of CDI patients visiting the ER.

The incidence of HA-CDI in South Korea has increased remarkably over the past 6 years (8). There is only one report about CA-CDI, but it concluded that CA-CDI comprised 10.5% of all CDI cases in South Korea (2). We found that among 32 CDI patients visiting the ER due to abdominal pain and diarrhea, 59.4% had CA-CDI. The age, antibiotic usage, and number of underlying diseases of CA-CDI patients were significantly lower than those of HA-CDI patients (Table 1; $P < 0.05$). Moreover, *C. difficile* isolation rates increased year by year from 2008 to 2010. Therefore, it is strongly recommended that physicians take a more careful medical history, including past hospitalization, and test for *C. difficile* in patients with diarrhea and other gastrointestinal symptoms to rule out CDI.

In our study, we had 3 cases of recurrent CDI. Two of these patients visited the ER about 1 week after discharge (case 10 and case 19). They developed diarrhea on the day of discharge. Similar to our study, 7 patients among 69 CO-HA-CDI patients actually had symptom onset prior to discharge, and 9 had symptom onset on the day of discharge (3). However, the other patient with recurrent CDI (case 35) was found more than 10 weeks after discharge, and the interval between the last diagnosis of CDI and recent symptom onset was 88 days. The patient had been hospitalized for DM and hypertension, after which she developed CDI. After recovery from CDI, the patient was admitted again due to endometrial cancer. At that time, no evidence of CDI was found. The case was classified in group B but was actually a recurrence case proven by rep-PCR and PCR-ribotyping and so should have been included in the CO-HA-CDI category. In group B, there were 3 other CDI cases without evidence of recurrence, and their symptom onset occurred as many as 6.5 to 8.5 weeks after discharge. In contrast, if the 13 CDI cases in groups A and B were considered hospital associated, the majority of patients (70%) would be identified less than 2 weeks after discharge. Other studies also have found that postdischarge follow-up of patients for 30 days identified 85% of cases and follow-up of patients for an additional 30 days would identify only an additional 4% of cases; the rates of 30-day CDI were not statistically significantly different from the rates of 60-day or 90-day CDI (3, 4). Therefore, it would be acceptable to define CO-HA-CDI if the case was discharged from hospital during the previous 12 weeks rather than to define indeterminate disease, although the majority of CO-HA-CDIs are found within 2 to 4 weeks after discharge.

Toxin A/B EIAs provided rapid diagnosis of CDI cases in the ER but were not fully helpful to investigate CDI cases visiting the ER. The low positivity of the EIA may have been due to the fact that the EIA kit for toxin A alone was used until 2007, and cases with A^-B^+ isolates might yield false-negative results (19). Compared to the toxin A/B EIA, *C. difficile* culture was a more sensitive method to detect CDI and was ordered more frequently since A^-B^+ isolates were reported to be highly prevalent in South Korea (16, 17, 18). Therefore, we recommended culture for *C. difficile* and other enteric patho-

gens concurrently to differentiate CDI and enteritis caused by other enteric pathogens.

With rep-PCR analysis, the diversity of patterns suggested little association among isolates from the patients visiting the ER with histories of hospitalization in the prior 10 weeks. Although 1 major and 4 minor clusters were observed, no relationships were found among them, including intervals of isolation (4 months to 6 years), location of the patient's address, or department of hospitalization. Of the 32 toxigenic isolates, A⁺B⁺ isolates were highly associated with CDI cases visiting the ER (78.1%), especially in group C (94.7%). The rep-PCR method correlated with PCR-ribotyping for A⁺B⁺ (12:18) and A⁻B⁻ (3:4) isolates but showed more diverse patterns in A⁻B⁺ (1:6) isolates. Pasanen et al. (14) reported that classifications by rep-PCR and PCR-ribotyping were comparable for 75% of isolates and the correlation of rep-PCR, PCR-ribotyping, and pulsed-field gel electrophoresis was excellent with the two major groups of isolates, PCR ribotypes 027 and 001. However, within PCR ribotype 001, rep-PCR was more discriminatory than PCR-ribotyping. Among other isolates, the grouping obtained with these three methods was less coherent. Rep-PCR could be more discriminatory than PCR-ribotyping within some ribotypes (like ribotypes 001 and 017), but grouping of some ribotypes into one rep-PCR group can also be detected (14).

A⁻B⁺ isolates were associated with HA-CDI cases (46.2% in HA-CDI cases and 85.7% in A⁻B⁺ isolates), especially in patients discharged within 2 weeks (55.6% in group A and 71.4% in A⁻B⁺ isolates). Only 1 A⁻B⁺ isolate was isolated from the nonhospitalization group, and it had a unique rep-PCR pattern. A⁻B⁺ isolates are highly prevalent in South Korea as nosocomial pathogens and are frequently associated with pseudomembranous colitis (16, 17). A⁻B⁺ *C. difficile* isolates may have been selected by particular antibiotic regimens used in hospitals or other unknown factors in South Korea. These findings suggest that A⁻B⁺ isolates still play a major role in HA-CDI but could also have a role in the etiology of CA-CDI. Therefore, the diverse rep-PCR patterns among A⁻B⁺ isolates were a remarkable finding of our study, and they might be helpful for epidemiologic studies and for revealing the relative virulence of A⁻B⁺ isolates because almost all A⁻B⁺ isolates showed the same ribotype pattern (ribotype 017) as in previous studies (16, 23). Stabler et al. suggested a low genetic diversity of the A⁻B⁺ isolates in their phylogenetic lineage study (21). It might be possible to determine the specific characteristics of A⁻B⁺ isolates which are more likely to spread nosocomially (via rep-PCR). No relationships were found among A⁻B⁺ cases, including intervals of isolation (1.5 months to 3.5 years), locations of the patients' addresses, or department of hospitalization. Both of the A⁻B⁺ isolates showing identical patterns (cases 30 and 31) were included in group A but had a 2.5-year interval of isolation between them.

In conclusion, the majority (70%) of CO-HA-CDI cases were detected within 2 weeks of the discharge, but the longest time interval to onset of symptoms after discharge was 10 weeks in CO-HA-CDI cases. Therefore, diarrheic patients visiting the ER with histories of hospitalization within 12 weeks are potentially affected by CO-HA-CDI. Overall, A⁺B⁺ isolates were highly prevalent in CDI cases visiting the ER, espe-

cially in presumed CA-CDI cases. A⁻B⁺ isolates were also associated with HA-CDI and may be emerging isolates in CA-CDI. Younger age, more limited underlying disease and antibiotic use histories, and genetic diversity of the isolates are the characteristics of the CA-CDI cases visiting the ER.

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